

# Development and validation of an HPLC-UV method for accelerated stability study and pharmacokinetic analysis of venlafaxine

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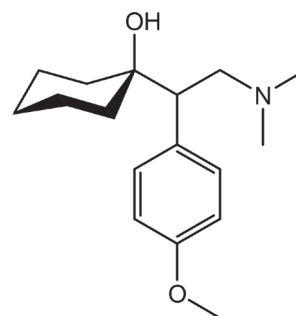
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A reverse phase high performance liquid chromatography method has been developed and validated for accelerated stability study and determination of pharmacokinetic parameters of venlafaxine HCl. The chromatographic separation was carried out using ODS analytical column (250 × 4.6 mm i.d., 5 µm particle size). The mobile phase included acetonitrile, methanol and potassium dihydrogen phosphate buffer (30:30:40; pH 6.1) at a flow rate 1.5 mL min<sup>-1</sup>. UV-Visible detector was used at wavelength of 227 nm to monitor elutions. Retention time observed was 2.745 min. The method was validated for linearity, accuracy, precision, sensitivity and robustness. Accelerated stability study of venlafaxine HCl capsules was carried out at 40 and 50 °C under 75% RH level. Suggested method was successfully applied for the pharmacokinetic analysis of venlafaxine hydrochloride tablets. Each of ten albino rabbits (≈ 1.2 kg each) was orally administered with 5 mg dose of venlafaxine HCl. The method was proved to be linear ( $R^2 > 0.998$ ), accurate (98.25-99.27%), sensitive (LOD: 35 ng mL<sup>-1</sup>; LOQ: 105 ng mL<sup>-1</sup>) and robust (RSD < 1%). The drug showed stability at accelerated conditions of temperature and humidity. The main pharmacokinetic parameters of tested products were as follows:  $t_{max}$  was 2.5h,  $C_{max}$  was 56.5 µg mL<sup>-1</sup>,  $t_{1/2}$  was 8.2 h,  $AUC_{0-36}$  was 845.9 µg h mL<sup>-1</sup>. The developed method is suitable to apply for quality control analysis and pharmacokinetic studies.

**Keywords:** Venlafaxine. HPLC. Stability. Pharmacokinetics. Chromatography.

## INTRODUCTION

Venlafaxine (IUPAC Name: 1-[2-Dimethylamino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanol, (Molecular formula: C<sub>17</sub>H<sub>27</sub>NO<sub>2</sub>, Mw: 277 g mol<sup>-1</sup>) (Figure 1) is a third generation, structurally novel phenethyl bicyclic antidepressant (Younus *et al.*, 2015). It was first introduced in 1993 by Wyeth. Venlafaxine is an important inhibitor of neuronal serotonin, norepinephrine reuptake and dopamine reuptake (Seshadri, Manohari, Kunchithapatham, 2013). In the gastrointestinal tract, absorption rate is about 92% but as a result of metabolism, its bioavailability is just 12.6% (Sánchez-Mata *et al.*, 2000). In liver, venlafaxine is rapidly metabolized by the action of CYP<sub>2D6</sub> into an active metabolite ortho-desmethylvenlafaxine (Mw: 263 g). Venlafaxine is highly water soluble drug (500 mg mL<sup>-1</sup>) (Asafu-Adjaye *et al.*, 2007).



**FIGURE 1** - Chemical structure of venlafaxine (1-[2-dimethylamino-1-(4-methoxy-phenyl)-ethyl]-cyclohexanol).

The most vital steps during drug development are pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of drug and formulated form of drug (Chan *et al.*, 2004). The common analytical techniques such as HPLC (Vu *et al.*, 1997; Titier *et al.*, 2003; Waschler *et al.*, 2004), LC-MS (Goeringer, McIntyre, Drummer, 2001; Juan, Zhiling, Huande, 2005;

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Bhatt *et al.*, 2005) and CE (Rudaz *et al.*, 2000), are utilized for pharmacokinetics and metabolism studies of venlafaxine. A number of HPLC methods (Makhija, Vavia, 2002; Bernardi *et al.*, 2009; Kaur *et al.*, 2010) have been reported in literature for pharmacokinetics and stability studies of venlafaxine (Liu *et al.*, 2011; Baldania *et al.*, 2008; Gursharanjit, Ghos, Dave, 2008). Determination of pharmacokinetics parameters for native drug and formulated form of the drug is utmost important to get real information about the bioavailability of newly developed/formulated drug. Therefore, we are interested to develop and validate HPLC-UV method for evaluation of venlafaxine hydrochloride for accelerated stability and pharmacokinetic parameters.

## EXPERIMENTAL

### Chemicals

Methanol (RCI Labscan), acetonitrile (Sigma-Aldrich) and acetic acid (Riedel-de Haën) were of analytical grade and no further purification was required.

### HPLC Analysis

HPLC analyses were carried out using Agilent 1200 series instrument (Agilent, Germany) equipped with a degasser (G1322A), diode array detector VL (G 1315B), quaternary pump (G 1311A), column compartment (G 1316A) and an auto sampler (G 1329A). Separation and quantification, of both Standard ( $1.0 \text{ mg mL}^{-1}$ ) and sample ( $0.75 \text{ mg mL}^{-1}$ ) solutions of venlafaxine HCl in mobile phase, were performed on a Shim-pack ODS ( $250 \times 4.6 \text{ mm i.d.}$ ,  $5 \mu\text{m}$  particle size) analytical column maintained at  $35^\circ\text{C}$ .

Most efficient separation conditions were achieved by employing different compositions of mobile phase during HPLC method development. A promising separation of drug was obtained by using a mobile phase mixture composing of  $\text{CH}_3\text{CN}$ ,  $\text{CH}_3\text{OH}$  and  $\text{KH}_2\text{PO}_4$  buffer in ratio of 30:30:40 with pH 6.1 (Table I).

### Method validation

The validation of an analytical method authenticates the parameters of the method for application (Parejiya *et al.*, 2014). The method used for the determination of venlafaxine HCl was validated for precision, accuracy, linearity, sensitivity and robustness. The developed method was validated according to the International Conference on Harmonization guidelines (ICH, 2005) and

**TABLE I** - Different compositions of mobile phase at pH 6.1

Mobile phase	Solvents		
	Acetonitrile:	Methanol:	Buffer
A	30:	30:	40
B	40:	30:	30
C	30:	40:	30

USP requirements (ICH, 1996)

### Accuracy and precision

Accuracy of analytical method is the proximity of the test results achieved by the method to the actual value. The precision is the extent of concurrence among the results of individual tests if the method is repeatedly applied to various samplings of standards (Chhalotiya, Patel, Bhatt, 2010). The accuracy and precision were calculated by using quality control samples which were prepared in three concentrations ( $50, 100, 200 \mu\text{g mL}^{-1}$ ) representing the whole range of standard curve (low, middle and high quality) of control sample. The response of each standard solution was measured in triplicate and the recovery percentage was calculated. The optimal chromatographic conditions were used to analyze the sample for ten times and it was followed calculation of mean.

### Linearity

The linearity of analytical method shows the direct proportionality of obtained test results to the concentration of analyte in sample within the given range (Chhalotiya, Patel, Bhatt, 2010). The linearity was evaluated by linear regression analysis. Quantitative analytical results are greatly affected by the quality of the calibration curve (Parejiya *et al.*, 2014). The calibration curve was obtained with seven concentrations of reference standard venlafaxine HCl solution ( $10, 50, 100, 200, 300, 400$  and  $500 \mu\text{g mL}^{-1}$ ) of the target analyte concentrations for the chromatographic method.  $20 \mu\text{L}$  of every concentration was injected in the chromatographic column through auto-sampler and allowed to separate.

### Sensitivity

For HPLC method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of calibration curve. Seven concentration levels except the blank were used to generate the calibration curve of venlafaxine HCl in the mobile phase. This curve was analyzed statistically to calculate the concentration of analyte in sample.

### Robustness

For the HPLC method, the robustness was determined by the analyses of the samples under a variety of conditions making small changes in the parameters like column temperature, buffer pH and mobile phase composition.

### Accelerated stability study

A novel and validated HPLC-UV method was applied to carry out accelerated stability studies of venlafaxine HCl capsules packed in gelatin shells placed in environment chamber (AC520, Perani, Italy). The study was carried out at 40 °C and 50 °C with 75% relative humidity during six months at various time intervals to determine any physical changes as well as their drug content. F-test was used to compare stability of two data replicates sets and calculate their respective precisions.

### Pharmacokinetic study

*In vivo* trial was conducted to determine the rate of absorption, distribution, metabolism and elimination of venlafaxine from the body. Ten male white albino rabbits ( $\approx 1.2$  kg each) were fasted overnight but allowed free access to water prior to the experiment.

All animals testing and experimental procedures were carried out under the guidelines mentioned in Good Laboratory Practice (GLP) regulations as described by United States Food and Drug Administration (USFDA) and Organization for Economic Co-operation and Development (OECD) Test Guidelines 425 (Up and Down Procedure) (Chen *et al.*, 2006). Before starting experiment, approval was taken from ethical committee of University of Sargodha, Sargodha, Pakistan (Ref. No. 20A28IEC UOS). The jugular vein of each rabbit was cannulated the day before drug administration. Venlafaxine HCl (5 mg) was orally administered to the rabbits. After administration, blood samples (3 mL) collected from the jugular vein cannula at 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10, 12, 24 and 36 h were kept in heparin containing tubes. The plasma samples were separated by centrifugation (3000 rpm, 5 min) using a refrigerated table top centrifuge and kept frozen at 0 °C until analysis.

The developed HPLC-UV method was used to analyze the plasma concentration of venlafaxine HCl. HPLC-UV analysis outcomes were plotted as mean plasma drug concentration vs. time. The pharmacokinetic parameters including  $t_{max}$ ,  $C_{max}$ , and AUC were calculated by non-compartmental analysis by Kinetica 5.0® computer software method.

## RESULTS AND DISCUSSION

### Optimization of solvent system

The mobile phase A (Table I) and standard solution of venlafaxine HCl were used to develop and validate an assay method for accelerated stability study of venlafaxine HCl. The pH and composition of mobile phase was varied to optimize the system. Figure 2 shows a standard chromatogram obtained from the analysis of a reference standard for venlafaxine HCl. The typical retention time (2.7 min with  $k'$  value 2.1) of venlafaxine HCl standard solution in mobile phase indicates that there is no drug-excipients interaction in the formulation (Kaur *et al.*, 2010), showing the efficiency of our validated method. The venlafaxine HCl peak was confirmed by spiking with the standard. The column is considered very efficient if the number of theoretical plates is more than 1500 (Sánchez-Mata *et al.*, 2000) that was found to be 1849.6 in our study. The tailing factor was found as 0.68, which is considered acceptable for chromatographic separations. The consistency of analytical results has great significance as it is required for proper analysis of pharmacokinetics results.

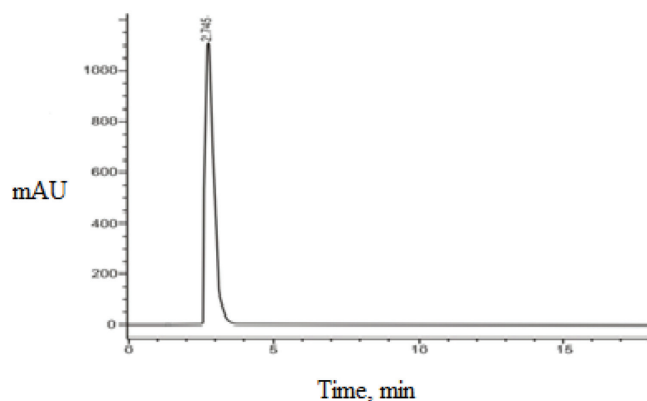


FIGURE 2 - Standard Chromatogram of venlafaxine HCl.

### Method validation

#### Linearity

The method was significantly linear for the drug in the concentration range of 10-500  $\mu\text{g mL}^{-1}$  (Figure 3). The plots represented the highest linearity ( $R^2 = 0.998$ ) revealing the good *in vitro* drug release of optimized formulation. The linearity was further confirmed by residual analysis, as a residual plot is a graph that shows the residuals on the vertical axis and the independent variable on the horizontal axis. If the points in a residual plot are randomly dispersed around the horizontal axis, a linear regression model is

appropriate for the data; otherwise, a non-linear model is more appropriate. As shown in the Figure 4, the random pattern indicates that a linear model provides a decent fit to the data. Table II shows the calibration curve data. The correlation coefficient for venlafaxine HCl obtained in this phase was 0.998 (Table IV).

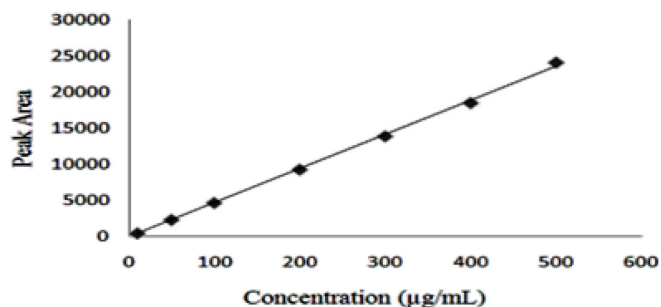


FIGURE 3 - Linearity Curve for venlafaxine HCl.

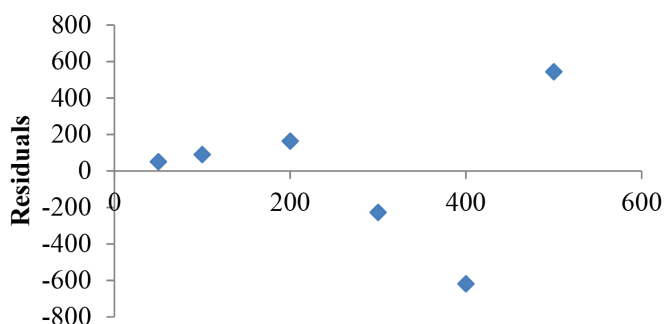


FIGURE 4 - Residual analysis of calibration curve.

#### Precision and accuracy

The validity of the planned method was assessed by recovery studies of standard addition method. Excellent recoveries (98.25-99.27%) were achieved for venlafaxine HCl indicating the method to be accurate. Percentage RSD was used to determine precision for intra-day and inter-day precision as shown in the Table III. The repeatability of newly developed method is proved by very low value of % RSD (Table V) confirming the method to be sufficiently precise under the same operating conditions.

TABLE II - Linearity data for venlafaxine HCl

Sr. No	Concentration (µg mL <sup>-1</sup> )	Peak area
1	10	397
2	50	2272
3	100	4618
4	200	9236
5	300	13854
6	400	18472
7	500	24090

#### Sensitivity

The sensitivity of developed method for quality control analysis of any drug is proved by LOD and LOQ values. For venlafaxine HCl, LOD and LOQ were found to be 35 and 105 ngmL<sup>-1</sup>, respectively (Table IV) that were higher than reported in literature (Chhalotiya, Patel, Bhatt, 2010).

#### Robustness

In all the deliberately varied chromatographic conditions, the chromatogram for system suitability solution showed satisfactory resolution. RSD < 1% with no significant changes in chromatographic parameters proves the robustness of newly developed method (Table V).

#### Accelerated stability study of venlafaxine HCl capsules

Figure 5 clearly shows the result of accelerated stability study of venlafaxine HCl capsules at 40 °C and 50 °C with 75% RH, at various time intervals. The concentration of venlafaxine HCl (the active ingredient) decreased slowly with R<sup>2</sup> = 0.9774 and R<sup>2</sup> = 0.9762 at 40 and 50 °C, respectively. The F-test results indicated that there is no significant difference in stability of venlafaxine HCl capsules at 40 and 50 °C with 75% RH. Stability study of the optimized formulation proved reliability of the drug tablets formulated by newly developed and

TABLE III - Intra-day and inter-day precision data for quantification of venlafaxine HCl

Concentration (µg mL <sup>-1</sup> )	Peak area of Venlafaxine HCl			
	Intra-Day		Inter-Day	
	Mean ± S.D.	% RSD	Mean ± S.D.	% RSD
50	3436 ± 13.03	0.3	3424 ± 18.02	0.5
100	4661 ± 18.36	0.4	4650 ± 31	0.67
200	9270 ± 13.66	0.15	9248 ± 28.03	0.30

**TABLE IV** - System suitability parameters for venlafaxine HCl

Parameters	Venlafaxine HCl
$\lambda$ max	227 nm
Linearity range ( $\mu\text{g mL}^{-1}$ )	10 – 500
Correlation coefficient ( $R^2$ )	0.998
Retention time (min)	2.7
Theoretical plates	1849.6
Tailing factor	0.68
Slope	47080
LOD ( $\text{ng mL}^{-1}$ )	35
LOQ ( $\text{ng mL}^{-1}$ )	105

validated HPLC-UV method, even after exposing to stress conditions of temperature and humidity.

#### Application of developed method (Pharmacokinetic study)

The precision, sensitivity and reproducibility of HPLC-UV method were good for pharmacokinetics

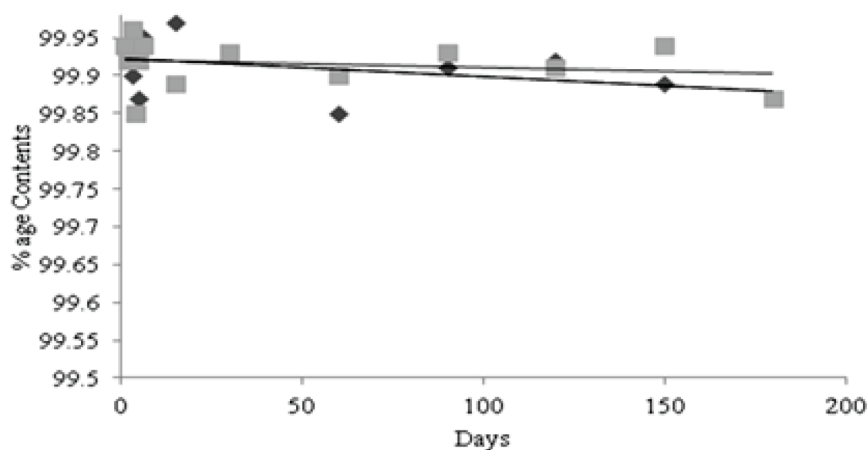
studies. The pharmacokinetic parameters were evaluated by single oral dose of 5mg venlafaxine hydrochloride capsule (dose as per body weight) to rabbits. The mean plasma concentration profile in oral single dose study is shown in Figure 6. Table VI presents the pharmacokinetic parameters. The  $C_{\text{max}}$  and  $\text{AUC}_{0-36}$  values are 56.5 and  $845.9 \mu\text{g h mL}^{-1}$ , respectively for venlafaxine tablet. These values corresponded to good bioavailability. The results indicate that the newly developed HPLC-UV method can also be used for the bioavailability study of venlafaxine HCl.

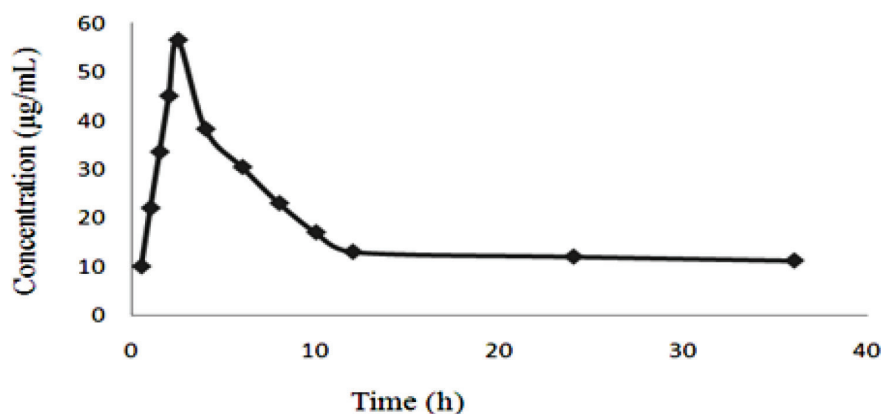
#### CONCLUSIONS

Newly developed and validated HPLC-UV method was successfully applied for accelerated stability and pharmacokinetic study of venlafaxine HCl. The method was proved to be efficient, robust, time conserving and offers good accuracy and precision when it is used to monitor full pharmacokinetic profile of venlafaxine HCl in rabbits. The main advantage of the method is that it uses small processing volume used for analysis and can therefore be employed for routine quality control analysis.

**TABLE V** - Robustness of HPLC-UV method

Drug	Parameters	Target conc. ( $\mu\text{g mL}^{-1}$ )	Mean conc. ( $\mu\text{g mL}^{-1}$ )	SD	% RSD	
Venlafaxine HCl	Wavelength	227 nm	9.5	0.102	0.110	
		228 nm	10	9.8	0.091	0.87
		229 nm		9.7	0.200	0.210
	Mobile Phase ( $\text{CH}_3\text{CN}:\text{CH}_3\text{OH}:\text{Buffer}$ ) (pH 6.1)	30:30:40		9.5	0.123	0.121
		40:30:30	10	9.7	0.244	0.246
		30:40:30		9.8	0.140	0.141

**FIGURE 5** - Stability curve showing the concentration of active ingredient in venlafaxine HCl capsules vs number of days at 40 °C & 75 % RH (■) and 50 °C & 75 % RH (◆).



**FIGURE 6** - Mean plasma concentration-time curve after a single oral dose of 5 mg venlafaxine HCl.

**TABLE VI** - Non compartmental pharmacokinetic analysis after a single oral dose (5 mg) administration

Pharmacokinetic parameters	Venlafaxine HCl (5 mg/animal)
$C_{max}$ ( $\mu\text{g mL}^{-1}$ )	56.5
$t_{max}$ (h)	2.5
$AUC_{0-36}$ ( $\mu\text{g h mL}^{-1}$ )	845.9
$t_{1/2}$ (h)	8.2
Cl ( $\text{mL min}^{-1}$ )	5.7
V (L)	40.5

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